Pharmacokinetics of pimobendan and its metabolite o-desmethyl-pimobendan following rectal administration to healthy dogs

by

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Abstract

Pimobendan, a benzimidazole-pyridazinone derivative, is an inodilator that is used for the treatment of congestive heart failure in dogs. Pharmacokinetics of pimobendan and its active metabolite o-desmethyl pimobendan (ODMP) were prospectively characterized in eight healthy dogs using a randomized, crossover design with a 24-h washout after a single dose of pimobendan (0.5 mg/kg) administered either per rectum (PR) or per os (PO). Plasma PIM and ODMP were quantitated using high performance liquid chromatography using an assay validated in dogs. Data were subjected to non-compartmental analysis. Pimobendan PR was more rapidly absorbed [time to maximum concentration (T_{max}) 1 ± 0.4 h] than PO (2.1 ± 0.9 h). Pimobendan was rapidly converted to ODMP within minutes after both PO and PR administrations. Plasma PIM and ODMP concentrations from pimobendan PR were found to be comparatively low at all time points compared to pimobendan PO. Pimobendan PR resulted in significantly lower C_{max} (PIM 10.1 ± 2 ng/mL, ODMP 8.8 ± 4.8 ng/mL) than pimobendan PO (PIM 49.1 ± 28.7 ng/mL, ODMP 30.9 ± 10.4 ng/mL). Relative bioavailability (%) of PIM and ODMP after rectal dosing was 25 ± 8 and 28 ± 6, respectively. Pimobendan PR was well tolerated by study dogs. Findings suggest that pimobendan PR might achieve effective concentrations and as such warrant future studies of clinical effectiveness in treating dogs with congestive heart failure who are unable to receive medication PO.

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List of Abbreviations

ACVIM: American College of Veterinary Internal Medicine

AUC: area under the concentration versus-time curve

CHF: congestive Heart Failure

CL/F: the ratio of clearance to bioavailability

C_{max}: maximum plasma concentration

C12: plasma concentration at 12 hours

F: relative bioavailability

HPLC: high performance liquid chromatography

LLOQ: the lower limit of quantification

MRT: mean residence time

ODMP: o-desmethyl-pimobendan

PDE3: phosphodiesterase 3

PIM: pimobendan

PO: per os

PR: per rectum

$t_{1/2}$: disappearance half-life

T_{max}: time to maximum concentration

V/F: the ratio of volume of distribution to bioavailability

1. INTRODUCTION

Pimobendan, a benzimidazole-pyridazinone derivative, is known as an inodilator that possesses the unique combination of positive inotrope and a vasodilator (1). It acts as a positive inotrope by sensitizing the affinity of calcium for binding to troponin C on cardiac myocytes and inhibiting phosphodiesterase 3 (PDE3). The inhibition of PDE3 also results in both arterial and venodilation. As a result, pimobendan improves cardiac output without increasing myocardial oxygen consumption (1–3). This unique combination of properties makes pimobendan desirable in the treatment of congestive heart failure (CHF) secondary to myxomatous mitral valve disease or dilated cardiomyopathy in dogs (2–5). The American College of Veterinary Internal Medicine (ACVIM) Specialty of Cardiology recommends pimobendan use for acute, hospital-based therapy of patients with current clinical signs of CHF as well as patients with advanced myxomatous mitral valve disease prior to the onset of CHF (ACVIM heart disease Stage B2, C, and D) (4).

The pharmacokinetics of oral pimobendan have been described (1,2). Pimobendan's active metabolite o-desmethyl-pimobendan (ODMP; UD-CG 212 Cl) contributes to pimobendan efficacy (2). Studies in conscious pigs have demonstrated that the metabolite is pharmacodynamically similar to the parent compound both qualitatively and quantitatively (6). In dogs, according to the approved product package insert, at 0.25 mg/kg tablet orally for the parent and active metabolite, the maximum drug concentration C_{max} (ng/ml) approximated 3 and 3.75, respectively at (T_{max}) of 1 and 4 hours; the terminal elimination or disappearance half-lives approximated 0.5 hours and 2 hours, respectively, for parent and active metabolite (7). Based on its pharmacodynamics, C_{max} and half-life, the active metabolite likely contributes to more than 50% of the bioactivity of pimobendan (6). After oral administration, bioavailability of

pimobendan in dogs is 70% (1,2); relative bioavailability of the active metabolite has not, apparently, been reported. Neither have been reported after rectal administration.

While the oral bioavailability of the commercial tablet is 70% (1,2,7,8), oral administration is not a viable route in an emergency situation. Many dogs presenting with advanced stage of CHF are in severe respiratory distress on presentation. Furthermore, some dogs are very fearful, or otherwise difficult to medicate per os. As such, oral administration may not be possible. For those scenarios, injectable pimobendan has been developed and used in multiple countries including Australia, Japan, and United Kingdom. When given intravenously, injectable pimobendan provides a rapid inotropic effect and decrease left ventricular end diastolic pressure in healthy dogs (9). However, to date, pimobendan is currently commercially available only as an oral, chewable tablet[a] in other countries including the United States. The manufacturer does not anticipate pursuing approval of any other pimobendan formulation in the United States. The lack of availability of alternative route of pimobendan limits its use in dogs in those countries where injectable formulation is not available.

Studies have shown that rectal administration can be a viable option for dogs who cannot receive medications per os (PO) (10-13). The potential advantages of rectal administration compared to oral administration include less stress for the patient, more rapid absorption, and decreased risk to the administrator in the case of patients who are otherwise unable to take oral medication (14,15). Due to the lack of injectable pimobendan, practitioners in the United States and other countries has anecdotally administered pimobendan per rectum (PR) in emergencies. To the authors' knowledge, rectal administration of pimobendan to dogs with CHF is supported only by anecdotal reports. Therefore, the efficacy or appropriate dose of pimobendan for rectal administration to dogs is unknown. In addition, there is a sparse data regarding the

pharmacokinetics of ODMP in dogs, despite findings suggest that ODMP may be a significant contributor to the hemodynamic effects of pimobendan (2,16).

The purpose of this study was to describe the pharmacokinetic characteristics of parent pimobendan (PIM) and ODMP following a single dose (0.5 mg/kg) oral or rectal administration in healthy dogs to explore the prospect of its clinical application. Our goal was to identify a therapeutic dose of pimobendan PR for dogs. This study aimed to obtain the relative bioavailability with a hypothesis that when pimobendan is administered rectally, pimobendan and ODMP will achieve plasma concentrations previously demonstrated to be therapeutic based on orally administered drug and that the increase in dose necessary to increase these concentrations will be less than 3 fold of the oral dose (7). Finally, we compared the pharmacokinetics of pimobendan following rectal administration as determined in this study to those following oral administration as determined in previous studies and package insert (7), to determine whether rectal administration might be an appropriate route for dogs in which oral administration of medications is limited.

2. MATERIALS AND METHODS

2.1. *Animals*

Eight privately owned healthy adult dogs (three males and five females) were included in the study. The sample size was designed to be able to consider a 40% difference in area of the curve being significantly different between the two routes (PO and PR), given a variability of 40% around the mean difference. Based on the maximum plasma concentration (C_{max}) and 90% confidence level, it was determined that eight dogs were necessary to describe a confidence interval that ranged 30% about the mean. Dogs included in the study were 3.7 ± 2.5 years of age

and weighing 26.8 ± 5.8 kg. All dogs were determined healthy based on benign medical history, normal physical examination, systolic blood pressure measured via Doppler technique, bloodwork (complete blood count and chemistry panel), and urinalysis. All dogs did not receive any drugs prior to and during the experiments other than preventative medication. During the study period, all dogs were housed individually in a ward designated for research within a veterinary teaching hospital. All dogs were observed for adverse effects for a period of 12 hours after drug administration and daily throughout the study period. All study protocols were approved by the institutional laboratory animal care and use committee, and informed consent was obtained from all owners before enrollment into the study.

2.2. Experimental design and drug administration

Dogs were studied using a randomized cross-over design with a 24 hours washout period between treatments. The washout period allowed for at least 10 half-lives of either PIM or ODMP to lapse between treatments to assure the previous dose was eliminated prior to administration of the second dose. Prior to study initiation, dogs were assigned a feeding schedule followed by withholding of food for 10 hours to standardize the transit time of luminal content as much as possible. Dogs were fed a commercial dry dog food during this study, except for not being fed for 10 hours before, and for 2 hours after pimobendan administration. The dose of pimobendan and the sampling times used in this study were based on a pilot study in 2 additional dogs for which a dose of 0.25 mg/kg PR resulted in concentrations that were insufficiently quantifiable to allow pharmacokinetic analysis (data not shown). As such, for this study dogs received pimobendan orally at 0.5 mg/kg, to the nearest tablet size (1.25, 2.5 or 5 mg Vetmedin tablet, Boehringer Ingelheim), with the rectal dose being the same (mg) as the oral

dose for each dog. Oral dosing was immediately followed by administration of 5 ml water by syringe over the base of the tongue for complete swallowing. For rectal administration, pimobendan suspensions were prepared a maximum of 30 minutes prior to the scheduled administration. The calculated dose of pimobendan tablet for each animal was finely crushed to assure adequate disintegration. Then the contents of the crushed tablet for each dog was prepared as a solution in 0.9% NaCl[b] by using 2 Luer Lock, 20-ml syringes connected to a 3-way stopcock to yield a pimobendan solution of 1 mg/mL. After mixing the entire suspension, and thus the entire dose for each animal and only for that animal, the solution was pulled up into a syringe, and the total dose was administered approximately 10 to 15 cm into the rectum in a 20 ml syringe, with a 16-inch (41 cm), 10 French red rubber tube[c]. To assure the entire dose was administered, the tube in which the dose was mixed was washed with a final 5 ml of 0.9% NaCl and this was used to flush the tube, thus assuring the entire calculated dose was administered to each animal. After the administration, the anus was digitally held closed for approximately 3 minutes to prevent early expulsion of the drug.

2.3. Sample collection

For all dogs, a 19 g, 16 or 25 cm central-line catheterd was placed in the saphenous vein for blood sampling, as described elsewhere (17). The catheter was "locked" with unfractionated heparin at a concentration of 200 U/mL. The catheter site was covered with sterile non-adherent dressing and secured with a wrap. An Elizabethan collar was placed on each cat to help prevent self-removal of the catheter. Twelve blood samples were obtained from the catheter immediately before drug administration (0) and at 5, 10, 15, 30, 45, 60, 120, 240, 360, 600, and 720 minutes thereafter, for each of the two study periods. Twelve hours after the final blood sampling was

collected following the first dose, dogs received pimobendan via the alternate route and the study was repeated as above. After collection of the final sample at 720 minutes, the IV catheter was removed, and the dogs were released home to their owner. All blood samples were collected into tubes containing lithium heparin[e] and placed over ice until processing. Plasma was separated within 1 hour by centrifugation at 27°C (3,500 x g, 10 minutes), and frozen separately at -70 °C until analysis.

2.4. Analytical Method

Plasma PIM and ODMP were quantified using high performance liquid chromatography (HPLC) with ultraviolet detection as previously reported with modifications. The HPLC system consisted of a Waters 717 plus auto sampler, Waters Binary pump 600 controller, and a 2487 UV-Visible detector (Waters Corporation™, Milford, MA, USA) (18). Briefly, separation was achieved with a Gemini C6, Phenyl 110A, 5 µm, 150 x 3.0 mm column (Phenomenex®, Torrance, CA, USA) at 40 °C (19). The mobile phase consisted of 0.6% Ammonium acetate buffer pH 3.0 and Acetonitrile (VWR®, Radnor, PA, USA) with the flow rate set to 0.8 mL/min (18,20). For sample preparation, briefly (8,18,19), 1000 µL of acetonitrile were added to tubes containing 500 µL of plasma. The contents of each tube were mixed vigorously through vortexing, then subjected to centrifugation for 10 minutes at 1900 x g at room temperature. The clear supernatant was transferred to a clean glass tube, the supernatant was evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted with 250 µL of mobile phase, vortexed for 20 sec, and then the solution was centrifuged for 10 min. 100 µL were injected into the column. The retention time for PIM was 6.0 min, and for ODMP was 3.0 min. The PIM and ODMP were detected with UV absorbance at 330 nm. Quantitation of PIM and ODMP were

based on the standard curves prepared in canine plasma containing known amounts of PIM (Sigma-Aldrich®, St. Louis, MO, USA) and ODMP (Cerilliant® Round Rock, Texas, USA). The standard curve concentrations ranged from 1 to 200 ng/mL for both PIM and ODMP. A standard curve was accepted if the coefficient of determination (r2) was at least 0.99 and the predicted concentrations were within 10% of the actual concentrations.

The linear correlation coefficient for PIM and ODMP standard curves were 0.998. The limit of detection (LOD) for PIM and ODMP in canine plasma was 0.5 ng/mL and 1 ng/mL respectively. The lower limit of quantification (LLOQ) for PIM and ODMP in canine plasma was 1 ng/mL and 2 ng/mL respectively. The Precision (CV %) for PIM in canine plasma 2, 14, 50 and 76 ng/mL was 2.49%, 1.80%, 2.74%, and 3.90% respectively. The Accuracy (% Recovery) for PIM in canine plasma 2, 14, 50 and 76 ng/mL was 100.10%, 103.43%, 105.86%, and 105.81% respectively. The Precision (CV %) for ODMP in canine plasma 2, 14, 50 and 76 ng/mL was 4.41%, 2.26%, 2.39%, and 4.23% respectively. The Accuracy (% Recovery) for ODMP in canine plasma 2, 14, 50 and 76 ng/mL was 101.40%, 104.75%, 103.09%, and 104.45% respectively.

2.5. Pharmacokinetic analysis

Plasma PIM and ODMP concentration versus time data was subjected to non-compartmental analysis using computer software[f]. Area under the concentration-versus-time curve from zero to the last time point (AUC) was determined using the log-linear trapezoidal method. The actual C_{max} occurring at time to maximum concentration (T_{max}) were recorded. Concentrations at 12 hours (C12) and at the last time point collected (C_{min}) were also recorded. The slope of the terminal component of the drug-elimination time curve was based on non-linear

regression. Because pimobendan was not given intravenously, the terminal component could not be confirmed to be elimination and thus both the terminal rate constant and corresponding half-lives were reported as disappearance; half-life ($t_{1/2}$) was reported as harmonic mean ± pseudo standard deviation. Furthermore, neither clearance (CL) nor volume of distribution (Vd) could be determined and are reported as to the ratio of either to bioavailability (Vd/F or CL/F). Other parameters included mean residence time (MRT) and the percent of the AUC that was extrapolated from the terminal component of the curve. The relative bioavailability of PIM and ODMP was estimated by AUC_{PR}/AUC_{PO}. The metabolite-parent AUC ratio was estimated by AUC_{ODMP}/AUC_{PIM}.

2.6. Statistical analysis

Statistical analyses were conducted by a commercially available spreadsheet[§]. The pharmacokinetic parameters were reported out as mean ± standard deviation. The Kolmogorov–Smirnov test was performed to evaluate the normality of parameters. The following comparisons were made between PO and PR routes, using a two-tailed paired t-test: C_{max}, T_{max}, and AUC. A t-test was used to compare the metabolite-parent AUC ratio between PO and PR. Values were considered significantly different at $P < 0.05$.

3. RESULTS

Pimobendan was well tolerated in all dogs receiving both routes. Authors did not observe the expulsion of pimobendan solution after rectal administration. No adverse effects were noted with both PR and PO pimobendan.

The mean of the actual doses administered was 0.51 (range 0.5-0.52) mg/kg for PO, 0.5 mg/kg for PR. One dog removed the central line catheter after completion of PR trial, and thus was excluded from the PO trial; PR data from this dog was retained. Mean ± SD log plasma drug concentration versus time plots of PIM and ODMP for either PO or PR are displayed in Figure 1. Mean pharmacokinetic parameters of PIM and ODMP in both routes are summarized in Table 1.

Plasma PIM and ODMP concentrations from pimobendan PR were found to be comparatively low at all time points compared to pimobendan PO. Significant differences in C_{max}, T_{max}, and AUC of PIM and ODMP were observed between PO and PR ($P < 0.05$; Table 1). Pimobendan PR resulted in significantly lower C_{max} (PIM 10.1 ± 2 ng/mL, ODMP 8.8 ± 4.8 ng/mL) than pimobendan PO (PIM 49.1 ± 28.7 ng/mL, ODMP 30.9 ± 10.4 ng/mL). Pimobendan was more rapidly absorbed via PR (T_{max} 1 ± 0.4 h) than PO (T_{max} 2.1 ± 0.9 h) ($P = 0.01$). The relative bioavailability (Relative F = AUC_{PR}/AUC_{PO}) of PIM and ODMP was 25 ± 8 and 28 ± 6, respectively, indicating that, AUC_{PR} was significantly lower than AUC_{PO} ($P < 0.05$).

Pimobendan was rapidly converted to ODMP after both PO and PR administrations because ODMP was detected in plasma within minutes (Fig. 1). The $t_{1/2}$ of PIM was shorter than ODMP in both routes. The concentration–time profile of ODMP lagged slightly behind that of PIM in both routes. The AUC_{ODMP} tended to be higher than the AUC_{PIM} in both routes. The metabolite-parent ratio (AUC_{ODMP}/AUC_{PIM}) was 1.46 ± 68 and 1.34 ± 1.25 for PO and PR, respectively. No statistical difference between PO and PR routes was detected ($P > 0.05$).

4. DISCUSSION

To the best of the authors' knowledge, this study is the first to describe the pharmacokinetic profiles of PIM and its active metabolite ODMP following rectal administration

in dogs. In the authors' experiences, dogs presenting with CHF requiring emergent treatment can be in severe respiratory distress, which may make the oral administration of the pimobendan challenging in the United States where tablet is the only form available. The size of pimobendan tablets are relatively larger compared to other tablets, which make it even more difficult to administer pimobendan PO especially to those patients. Furthermore, there are scenarios when dogs in respiratory distress vomit after medication is administered PO, which makes the absorption questionable to achieve therapeutic concentration (21). For those scenarios, pimobendan PR may provide an option for short-term therapy for dogs as their first dose. This study demonstrated that the pimobendan PR can achieve a presumed therapeutic plasma concentration of PIM and ODMP in healthy dogs based on the pharmacokinetic profiles provided in the package insert (7). Furthermore, pimobendan PR showed similar metabolite-parent AUC ratio (AUC_{ODMP}/AUC_{PIM}) compared to its oral administration. Therefore, the findings in this study suggests that the use of pimobendan PR may achieve therapeutic plasma concentrations similar to pimobendan PO.

To determine the pharmacokinetic profile and relative bioavailability of pimobendan PR, this study used a dose that was two times greater (0.5 mg/kg) than that routinely used in oral administration (0.25 mg/kg) (4). Pimobendan decreases left atrial pressure as a dose dependent manner and thus the higher dose (0.5 mg/kg) potentially may be beneficial as short term therapy for dogs with CHF (22,23). Previous pharmacokinetic studies in dogs suggested that the dosage for rectal administration should be higher dose than oral administration based on the relatively lower bioavailability, C_{max} and AUC compared to those from oral administration (11–13,24,25). Possible reasons for this include relatively small surface in dogs' rectum available for drug uptake, the presence of feces sequestrating drugs in the rectum at the time of administration, or

the possibility of inadvertent expulsion of drugs after administration (14,15,25). Similar to the previous studies, pimobendan PR showed significantly lower AUC and C_{max} compared to oral administration ($P < 0.05$). Furthermore, in order to have sufficient data points to determine the relative bioavailability of pimobendan PR, the authors determined that 0.5 mg/kg dosing was necessary to be able to have sufficiently high plasma drug concentrations to allow quantitation. Pimobendan 0.5 mg/kg PR was well tolerated in this study with no obvious signs of gastrointestinal discomfort or other physiologic changes. This study is not designed to evaluate potential adverse effects of long-term administration of pimobendan at a dose of 0.5 mg/kg because the PR route will benefit dogs receive single dose during initial stabilization.

In this study, the authors determined that 0.9% NaCl was appropriate as a drug delivery because of its accessibility in the clinical practice in comparison to specifically formulated suppositories. In addition, the dogs in the present study did not receive an enema or manual evacuation of fecal material prior to the PR administration of pimobendan solution. These methods we used can be followed by any veterinarian with no specialized equipment. Solutions tend to be absorbed more quickly through rectum, produce effects more rapidly, and may not avoid first-pass metabolism compared to other formula (14,15,26). Although the risk of impaired absorption due to the presence of feces, we chose not to manually evacuate feces or perform enema, in order to better approximate the conditions in which pimobendan PR will be used in clinic. This is clinically important, as dogs requiring pimobendan PR might have fecal material in the rectum at the time of administration.

This study demonstrated that the rectal administration of pimobendan (0.5 mg/kg, PR) can achieve the plasma concentration of PIM and ODMP in healthy dogs higher than those provided in the package insert (7). Provided that the plasma concentrations of PIM and ODMP in

the package insert produce desired hemodynamic effects in dogs, analysis of results of our study suggests that the higher dose of pimobendan PR could potentially be an alternative to PO. However, the therapeutic efficacy of the plasma concentrations reported in our study are difficult to interpret, partly because plasma concentrations associated with presumed efficacy of PIM and OMDP are not clearly defined in dogs. The authors noticed a disparity among the pharmacokinetic profiles of pimobendan reported by others (1,7,8,23). The key pharmacokinetic parameters of PIM and ODMP from different studies are summarized in Table 2. Pimobendan capsule 0.25 mg/kg resulted in a C_{max} of 38.1 ± 18.3 ng/mL(1). In two other studies, both using pimobendan suspensions (but different products), 0.27 mg/kg of a pimobendan suspension produced C_{max} of 18.6 ng/mL (6.1-25.3) yet 0.3 mg/kg resulted in a C_{max} of 7.3 ± 2.7 ng/mL (8,23). This disparity may be due to the differences in the design of the experiment (e.g. dogs participated in each study, analytical method) or the effects of different formulation of drug absorption. Therefore, the clinical usefulness of pimobendan administered PR in the present study warrants further investigation combined with pharmacodynamic investigation to determine the correlation between plasma concentrations and the cardiovascular effects.

Notable findings of this PK study include that pimobendan appears to be more rapidly absorbed when it was administered PR than PO based on T_{max} which was significantly shorter for PR for both PIM and ODMP (1 to 1.5 hours earlier, respectively) compared to the PO route ($P = 0.01$ for both PIM and ODMP). The more rapid absorption following rectal administration has the potential to be advantageous over oral administration as it reduces the lag time from treatment to effect in dogs with severe respiratory distress. In addition, pimobendan was more rapidly converted into its metabolites ODMP. When administered PO, pimobendan undergoes the first pass hepatic metabolism via oxidation and converted into ODMP (2,16). It is known that

the PDE3 inhibitory action of ODMP is significantly more potent than that of parent pimobendan (8,16,20,26). Endoh et al reported that ODMP is 500 times more potent of an inotrope compared to the PIM in isolated canine ventricular muscle (16). Therefore, ODMP is active and may have an important contribution to the hemodynamic effects of pimobendan. However, there is a sparse data regarding the pharmacokinetic profile of ODMP in dogs (28). The manufacturer's package insert reported that pimobendan 0.25 mg/kg PO resulted in an ODMP C_{max} of 3.66 ± 1.21 with T_{max} 2 hours (7). Yata et al reported when nonaqueous solution of pimobendan 0.27 mg/kg was administered PO, pimobendan was rapidly converted to ODMP and the systemic exposure was greater for ODMP than for PIM due to a slower elimination (8). These findings are similar to the results in our study.

An important aspect of rectal administration is that the possibility of partial avoidance of hepatic first pass metabolism (14,15). This is dependent on how far caudally the drug is deposited within the rectum (26). If the drug is administered in the lower part of rectum, drugs absorbed may bypass the liver and be delivered directly to the systemic circulation, whereas drugs absorbed to the proximal portion of the rectum is drained by the portal vein and still undergo first pass metabolism (15). First pass metabolism becomes more clinically important if the parent compound is metabolized to an active metabolite, as is the case with pimobendan. Analysis of our findings suggest that pimobendan PR does not appreciably avoid first-pass metabolism in dogs. The metabolite-parent AUC ratio (AUC_{ODMP}/AUC_{PIM}) following pimobendan PR was similar to that of pimobendan PO. However, dogs' rectums are approximately 5-cm long, and 2.5 cm of the rectum drains into the systemic circulation (26). Furthermore, it is likely that the length of rectum will vary significantly depending on the breed and size of each individual. Therefore, it is highly likely that pimobendan administered PR was

absorbed to the portions of the rectum that do not bypass the portal system and thus PIM was converted to ODMP in a similar magnitude following rectal administration. Therefore, the findings in our study showed potential benefits of pimobendan PR based on more rapid absorption compared to PO, and similar magnitude of first pass metabolism. Further studies will need to include more animals with sampling at more frequent time intervals to more fully characterize the pharmacokinetic profile after rectal administration in dogs.

A limitation to this study includes the lack of injectable pimobendan in addition to oral and rectal dosing. Unfortunately, pimobendan is not available for intravenous administration in the United States and the manufacturer does not anticipate pursuing approval of any other pimobendan. Bioavailability is most accurately determined by comparing AUC measurements from intravenous and non-intravenous administration of a drug. Although it may not present the most the precise pharmacokinetics data, this study provides a powerful insight to the pharmacokinetic profile of rectally administered pimobendan. The result of this study shows the efficacy of rectal route to complement oral administration of the tablet form in medical emergencies. An additional limitation to this study is the fact that healthy dogs were used. The dogs used in this study were considered healthy based on history, physical examination and clinicopathological findings. Using healthy animals is often necessary in such studies. On the other hand, when rectal dosing is used in the clinical setting, this route will commonly be administered to dogs with CHF with decreased cardiac output leading end-organ hypoperfusion. It is possible that due to the reduced cardiac output, age, concurrent treatments, or other comorbidities, the bioavailability, metabolism, or excretion of pimobendan and could be altered in dogs who will receive benefit from this route. Another concern is that dogs also may expel an unmeasurable amount of the drug following rectal administration. However, it is important to

note that the expulsion of medication administered PR can make it difficult for the practitioner to determine how much drug has been absorbed. Last limitation of this study is that the final concentration of pimobendan solution prior to administration was not measured. The methods used to prepare the pimobendan solution in this study were designed to mimic techniques that has been used in clinics. In the pilot study, analysis of pimobendan solution prior to administration showed that the concentration of pimobendan solution administered was consistently 30% less than the intended concentration. Therefore, the actual dose of pimobendan in the administered solution in the pivotal study could be lower than the intended dose. As such, the bioavailability reported in the current study can be lower than the one from expected concentration.

5. CONCLUSION

In conclusion, the bioavailability of pimobendan following rectal administration was lower compared to the oral administration. We conclude that the rectal administration of a single dose (0.5 mg/kg) pimobendan achieved presumably therapeutic plasma concentration in dogs based on the package insert. Given the rapid absorption to achieve maximum concentration in conjunction with the metabolite-parent AUC ratio similar to oral administration, the rectal administration of pimobendan may be a suitable option for immediate stabilization for dogs with CHF. Additional studies need to be performed to determine the recommended therapeutic target range of plasma concentration of PIM and ODMP in dogs. Further studies are warranted to describe the pharmacokinetics of pimobendan following repeated dosing in conjunction with pharmacodynamics investigation for dosing recommendation for this route.

Table 1. Summary of pimobendan and O-demethylated-metabolite (ODMP) pharmacokinetic parameters (mean ± SD) for a single dose of pimobendan in dogs.

	Pimobendan PO		Pimobendan PR		*P* value (PO vs PR)	
Parameter	PIM	ODMP	PIM	ODMP	PIM	ODMP
C_{max} (ng/mL)	49.1 ± 28.7	30.9 ± 10.4	10.1 ± 2	8.8 ± 4.8	0.002	0.0001
C_{min} (ng/mL)	2.5 ± 1.4	2.8 ± 1.1	1.7 ± 0.9	1.7 ± 0.5	ND	ND
C12 (ng/mL)	2.4 ± 1.6	5.2 ± 2.5	1	3.1 ± 1.3	ND	ND
T_{max} (h)	2.1 ± 0.9	3.2 ± 1.6	1 ± 0.4	1.7 ± 1.1	0.01	0.01
$t_{1/2}$ (h)	1.8 ± 0.8	5.0 ± 2.7	2.2 ± 0.6	8.3 ± 4.8	> 0.05	> 0.05
AUC (ng*h/mL)	148.4 ± 71.6	167.8 ± 36.2	31.1 ± 11.9	50.1 ± 19.2	0.03	0.0006
MRT (h)	3.9 ± 1.3	8.3 ± 3.5	3.5 ± 0.9	13.2 ± 8.0	> 0.05	> 0.05
CL/F (mL/h/kg)	0.004 ± 0.002	0.003 ± 0.001	0.012 ± 0 .003	0.010 ± 0.005	ND	ND
V/F (mL/kg)	0.011 ± 0.009	0.022 ± 0.013	0.036 ± 0.002	0.120 ± 0.074	ND	ND
F (AUC_{PR}/AUC_{PO}, %)			25 ± 8	28 ± 6		

AUC, area under the concentration versus-time curve; CL/F, the ratio of clearance to bioavailability, C_{max}, maximum plasma concentration; C_{min}, concentration at the last time point collected; C12, plasma concentration at 12 hours; F, relative bioavailability; MRT, mean residence time; ND, not determined; ODMP, o-desmethyl-pimobendan; PIM, pimobendan; PO,

per os; PR, per rectum; $t_{1/2}$, disappearance half-life; T_{max}, time to maximum concentration, V/F; the ratio of volume of distribution to bioavailability

Table 2. Data of previous studies investigating the pharmacokinetic properties of pimobendan in dogs [all presented as arithmetic mean ± SD except for Yata et al. presented as (median, range)]

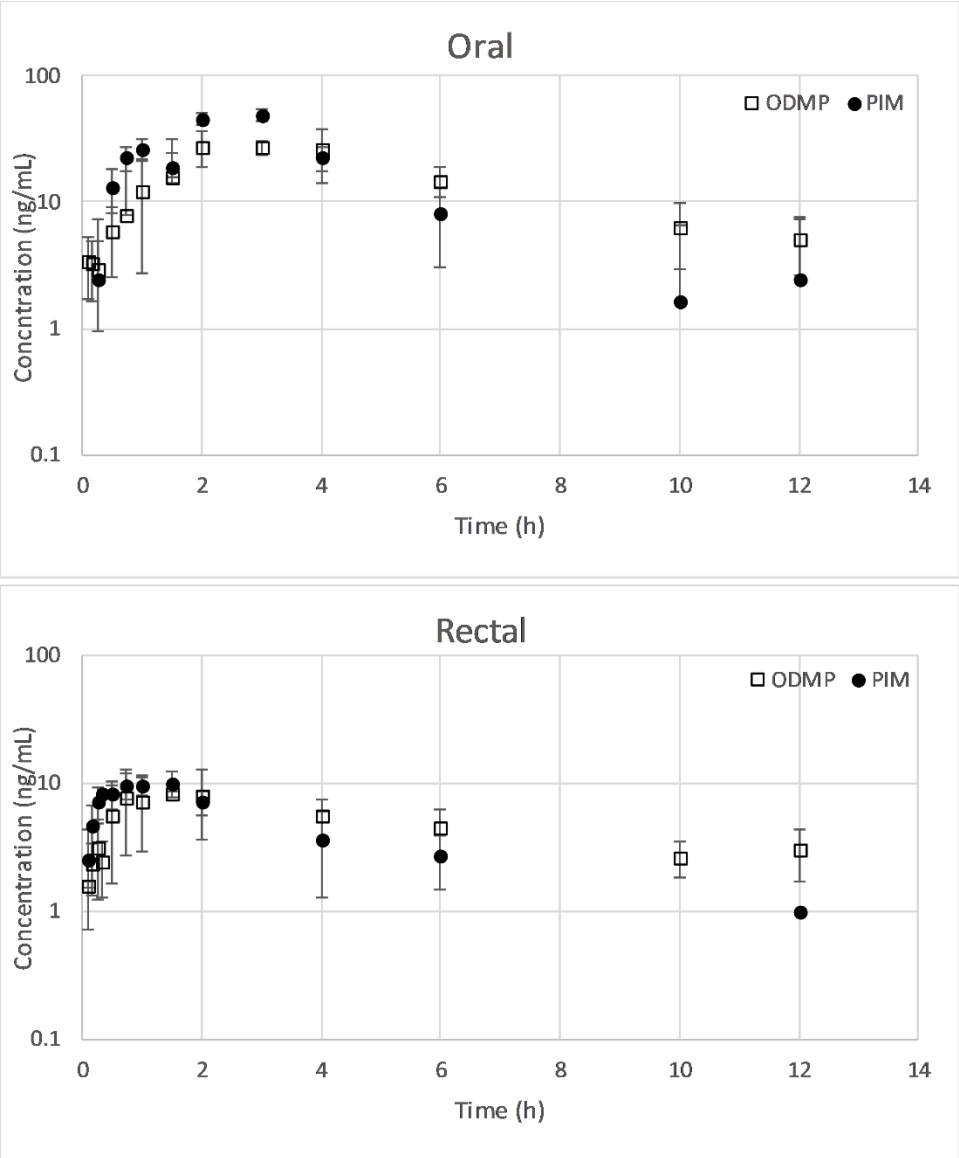
	Bell et al. 2015			Yata et al. 2015		Package insert		Her et al. 2020				Guth et al. 2015
Formulation	Capsule	Solution	IV	Solution		Tablet		Tablet		Solution		Suspension
Route	PO		IV	PO		PO		PO		PR		PO
Dose (mg/kg)	0.25		0.125	0.27		0.25		0.5		0.5		0.3
	PIM			PIM	ODMP	PIM	ODMP	PIM	ODMP	PIM	ODMP	PIM
C_{max} (ng/mL)	38.1 ± 18.3	39.4 ± 23.4	51.1 ± 28.5	18.6 (6.1-25.3)	16.2 (6.0-22.3)	3.09 ± 0.76	3.66 ± 1.21	49.1 ± 28.7	30.9 ± 10.4	10.1 ± 2	8.8 ± 4.8	7.3 ± 2.7
T_{max} (h)	N/A	N/A	N/A	1.1 (0.5-2.0)	1.3 (0.8-2.0)	2	3	2.1 ± 0.9	3.2 ± 1.6	1 ± 0.4	1.7 ± 1.1	3.2 ± 1.3

AUC (ng*h/mL)	N/A	N/A	N/A	27.1 (15.2-44.2)	42.1 (25.1-52.7)	N/A	N/A	148.4 ± 71.6	167.8 ± 36.2	31.1 ± 11.9	50.1 ± 19.2	22.5 ± 10.4
$t_{1/2}$ (h)	N/A	N/A	N/A	0.9 (0.7-1.1)	1.6 (1.3-1.9)	N/A	N/A	1.8 ± 0.8	5.0 ± 2.7	2.2 ± 0.6	8.3 ± 4.8	N/A
F	N/A	N/A	N/A	N/A	N/A	N/A	N/A			25 ± 8	28 ± 6	N/A
LLOQ (ng/mL)	2.5	2.5	2.5	0.5	0.5	N/A	N/A	1	2	1	2	N/A
Analytical method	LCMS	LCMS	LCMS	UHPLC-MS	UHPLC-MS	N/A	N/A	HPLC	HPLC	HPLC	HPLC	N/A

AUC, area under the concentration versus-time curve; C_{max}, maximum plasma concentration; F, relative bioavailability; LCMS, normal-phase liquid chromatography-mass spectrometry analysis assay; LLOQ, lower limit of quantification; ODMP, o-desmethyl-pimobendan; PIM, pimobendan; PO, per os; PR, per rectum; $t_{1/2}$, disappearance half-life; T_{max}, time to maximum concentration, UHPLC-MS, ultra-high-performance liquid chromatography–mass spectrometer assay

Figure 1. Mean + SD of concentration–time plots of PIM and ODMP for dogs treated with a single dose of 0.5 mg/kg pimobendan PO (n = 7) versus PR (n = 8).

ODMP, o-desmethyl-pimobendan; PIM, pimobendan



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FOOTNOTES

a. Vetmedin, Boehringer-Ingelheim, Ingelheim, Germany.

b. 0.9% NaCl, Sagent Pharmaceuticals, Schaumburg, Ill.

c. Feeding Tube and Urethral Catheter, 10 Fr, Kendall, Tyco Healthcare Group LP, Mansfield, Mass.

d. MILACATH 19 g, 25 cm intravenous catheter, Item # PI1910; Mila International, Inc, Erlanger, KY

e. Monoject lithium heparin tubes, Fisher Scientific, Itasca, Ill.

f. Phoenix WinNonlin, version 7.0, Certera Corp, Princeton, NJ.

g. Microsoft Office 2010. Microsoft Excel (version 14). Microsoft Corporation, 2010, Redmond, WA